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TECHNICAL MANUSCRIPT 2

PRACTICAL PROCEDURES

FOR

MICROBIAL DECONTAMINATION

MARCH 1962

U.S. ARMY CHEMICAL CORPS
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY CHEMICAL CORPS RESEARCH AND DEVELOPMENT COMMAND
U.S. ARMY BIOLOGICAL LABORATORIES
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FOREWORD

Too often, in the infectious disease laboratory, routine disin-
fection or decontamination procedures are applied in a haphazard
and ineffective manner. This is often due to a lack of understand-
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This report discusses some practical procedures that are readily
applicable to any infectious disease laboratory.

Appreciation is due Dr. Dorothy G. Smith for her valued assist-
ance in the preparation and editing of this report.

ABSTRACT

Routine disinfection or decontamination procedures in the
infectious disease laboratory are too often applied in a haphazard
and ineffective manner. This is often due to a lack of under-
standing of the limitations of various methods of decontamination.
This report discusses some practical procedures that are readily
applicable to any infectious disease laboratory. These techniques,
employing heat, chemicals (liquids and vapors), and radiation
result from experimentation and extensive use in laboratories
working with a variety of infectious disease agents.

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I. INTRODUCTION

Methods and principles of sterilization have been among the most actively discussed and investigated areas in the field of microbiology. They also comprise an area of knowledge that is vital to any serious work involving microorganisms. In spite of the extensive literature on sterilization, practical problems continue to arise for anyone employing routine sterilization procedures—problems for which there may be no clear-cut answer. This seeming dilemma arises from the fact that heat, the most reliable means of sterilization, cannot be applied in many situations arising in an infectious disease laboratory or similar installation. It therefore becomes necessary to resort to less reliable means of sterilization, or to settle for something less than sterilization, which we may call disinfection or decontamination.

It has long been recognized that chemical decontamination is made difficult by the existence of species differences in susceptibility. In addition, the velocity of the process of sterilization by different chemicals depends to a variable degree on dilution, temperature, presence of organic matter, hydrogen ion concentration, extent of penetration, surface tension, and other environmental factors. The application of germicidal radiation such as ultraviolet light is limited by its low penetrating power. Therefore, valid data must be based on the results of experiments imitating any particular anticipated environmental condition for each particular species of microorganism.

In spite of these recognized difficulties in formulating generally valid rules for decontamination, some principles of decontamination will be discussed and some germicides and procedures listed that have been used sucessfully under practical conditions. It must be realized that any of the conclusions reached are supported only by results of experiments with relatively few species of spore-forming and nonspore-forming microorganisms. However, the procedures given are primarily those that have been found to be useful in any laboratory in which work is conducted with infectious disease agents.

II. DECONTAMINATION METHODS

Of the numerous physical and chemical means of sterilization or inacti-
vation of microorganisms, those that are most applicable to the infectious
disease laboratory may be classified under one of four main headings: (a)
heat, (b) liquid decontaminants, (c) vapors and gases, and (d) radiation.

A. HEAT

It is generally accepted that the application of heat, either dry or
moist, is the most effective method of inactivating microorganisms. The
exposure temperatures and times required for sterility are known and can be
readily controlled. Whenever possible, heat should be used to sterilize
materials.

B. VAPORS AND GASES

A variety of vapors and gases possess germicidal properties. Among these
are formaldehyde, ethylene oxide, methyl bromide, ethyleneimine, beta-propio-
lactone, and peracetic acid. When these can be employed in closed systems
and under controlled conditions of temperature and humidity, excellent decon-
tamination can result. Formaldehyde, peracetic acid, and beta-propiolactone
can be easily dispensed and controlled for the decontamination of rooms or
buildings. Formaldehyde is persistent and difficult to remove after use, and
peracetic acid is corrosive for metals and rubber. Beta-propiolactone holds
great promise as a space decontaminant. In the vapor form, it acts rapidly
against bacteria,[1][*] rickettsiae, and viruses,[2,3] has no adverse effect on
most materials, and disperses rapidly after use, leaving no residual fumes
or odors.

Under controlled conditions, ethylene oxide is an effective sterilizing
agent, convenient to use, versatile, and noncorrosive.

C. LIQUID DECONTAMINANTS

There are probably more misconceptions in circulation concerning the use
of liquid decontaminants than of all other types combined. This is largely
due to a characteristic capacity of such liquids to perform dramatically in
the test tube and to fail miserably in a practical situation. Such failures
often occur because too little consideration is given to such factors as
temperature, contact, pH, concentration, and the presence of organic material
at the site of application. Small variations in the above factors may make
large differences in germicidal effectiveness. For this reason, even when

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[*] See Literature Cited.

used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants. For example, even though contaminated pipettes are discarded into a disinfectant solution, pipettes and solution should be autoclaved before being handled by dishwashers.

Hundreds of decontaminants or germicides are available under a variety of trade names. Most, however, may be classified as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydic compounds and other organic preparations. None are equally useful or effective under all conditions.

In the decontamination of large areas or rooms after accidental spills, etc., the mechanical removal of microorganisms by washing with water or disinfectants plays an important part. For this reason, surface-active agents[4] are often incorporated in germicidal solutions. The most frequently used liquid disinfectants in infectious disease laboratories are chlorine solutions, phenol and related acids, mercuric chloride, formalin, quaternary ammonium compounds, and sodium hydroxide solutions. Solutions of soap must not be overlooked for decontamination purposes.

Table I lists some commonly used germicides with recommended exposure times, concentrations, and types of pathogens believed to be susceptible. All exposures are assumed to be at a temperature of approximately 25°C.

When decontamination with chemical solutions is required, viruses and rickettsiae present special problems. The evaluation of the virucidal and rickettsicidal action of chemicals is difficult. Most tests take place under various conditions of time, temperature, pH, and organic material that may be hard to duplicate. Complete inactivation is difficult to determine because of the methods used for virus detection and assay. In addition, information on virus inactivation frequently is the coincidental result of experiments carried out to determine immunological activity of the virus after treatment with various chemicals. The concentrations of chemical and exposure times used are often not those that would be desirable for practical decontamination. It is often stated that the reaction of certain chemicals with specific viruses follows the course of a reaction of the first order. However, recent experience with formaldehyde-inactivated poliomyelitis virus vaccine has clearly demonstrated the pitfalls encountered when extrapolated data are used to determine sterility.[5]

A similar occurrence with Venezuelan equine encephalomyelitis virus at Fort Detrick further emphasizes this point. Several infections followed the use of a "live" vaccine because of inadequate inactivation of the virus by formalin.[6]

Some recommendations for practical chemical disinfection of viral and rickettsial suspensions are shown in Table II. These recommendations were derived from a number of experiments designed specifically to determine the conditions necessary for chemical disinfection,[2,3,7] as well as from considerable practical experience.

TABLE I. RECOMMENDED CONDITIONS FOR USE OF COMMON GERMICIDES
AT ROOM TEMPERATURE (25°C)

GERMICIDE	CONCENTRATION AND EXPOSURE TIME Typical Classes of Microorganisms			
	Vegetative bacteria such as Salmonella Brucella Pasteurella	Bacterial spores such as B. anthracis	Fungi such as Blastomyces Histoplasma Coccidioides	Bacterial toxins such as Botulinum Staphylococcus
Phenol	5%, 5 min.	NR[a/]	5%, 15 min.	NR
Lysol	2%, 5 min.	NR	3%, 15 min.	NR
Quaternary Compounds (Roccal, Purasan, Hyamine, etc.)	0.1 to 1.0% 5 min.	NR	NR	NR
Hypochlorites plus 1% Wetting Agent (Naccanol, etc.)	200 to 1000 ppm, 1 min.	500 to 5000 ppm, 5 min.	2000 ppm, 10 min.	NR
Caustic Sodium Hydroxide	2%, 15 min.	5%, 30 min.	10%, 30 min.	5% [b/] (pH 11.5) 15 min.
Formalin (37% HCHO)	5%, 10 min.	10%, 10 min.	5%, 10 min.	5%, 10 min.
Steam-Formaldehyde Vapor (closed areas)	1 ml per cu ft in air with RH above 80%, 30 min.			NR
Beta-Propiolactone Vapor (closed areas)	200 mg per cu ft in air with RH above 80% 30 min.			NR
Ethylene Oxide Gas	300 mg per liter for 8 to 16 hours			NR

a. Not recommended.
b. Staphylococcal enterotoxin requires 24 hours' exposure at room temperature,
   90 minutes' at 210°F.

TABLE II. RECOMMENDATIONS FOR PRACTICAL CHEMICAL DISINFECTION OF VIRAL AND RICKETTSIAL SUSPENSIONS WITHIN 30 MINUTES AT 25°C

CHEMICAL	RICKETTSIAE OF		VIRUSES OF					
	Q Fever	RMSF	Psittacosis	VEE	JBE	Yellow Fever	Smallpox	Rift Valley Fever
DECONTAMINATION WITH SOLUTIONS								
Lysol, %	1*a/	1*	5	NRc/	1*	5*	1*	5
Quaternary Ammonium Compounds, Roccal, Purasan, etc., %	-b/	2*	2*	2*	2	2*	2	-
Sodium Hydroxide (NaOH), %	-	5	-	5NT	5NT	-	1	-
Formalin, %	10NTd/	10NT	1	10NT	10NT	10NT	2	10NT
VAPOR PHASE DECONTAMINATION								
Steam-Formaldehyde	1 ml per cubic ft of air with RH above 80% for 1 hr						R-NTe/	R-NT
	*	*	*	*	*	*		
Ethylene Oxide, 300 mg per liter	Freoxide or Carboxide at 20 pounds pressure for 12 hours						NR	-
	*	R-NT	*	*	-	-		
Beta-Propiolactone, 300 mg per ft³ of air	RH above 90% for 30 min						*	*
	*	R-NT	*	*	*	*		
SOLID DISINFECTANT FOR USE IN PACKAGING AGENT								
Sodium Hydroxide Pellet	R-NT	*	R-NT	R-NT	R-NT	(Citric acid crystals are recommended.)	*	-

a. Asterisk designates disinfectant of choice.
b. Insufficient information.
c. Not recommended.
d. Not tested.
e. Recommended but not tested.

Notes on Table II

1. Where appropriate (as indicated in Table II), it is suggested that Lysol be used as a 5 per cent solution, Roccal as 2 per cent, and formalin as 10 per cent.

2. Three cautions must always be borne in mind:

    (a) Chemical disinfection may not be equivalent to complete sterilization.

    (b) Crude menstrua and low temperatures may retard virucidal action.

    (c) Specific tests should be performed whenever dependence is to be placed routinely on chemical disinfection.

3. The practical recommendation for use, for use as disinfectant of choice, or for nonuse (NR-not recommended) is made on the basis of direct or conservatively extrapolated experimental data. In some cases, the recommendation is based on considerable practical experience but without specific experimental testing (R-NT-recommended, not tested). In other cases the entry simply indicates a lack of sufficient data or experience on which to base a conservative opinion. Conditions of menstruum, time, temperature, relative humidity, and disinfectant concentration are particularly important in vapor phase decontamination and any general statement must be taken with caution.

D. RADIATION

In certain specific applications, germicidal ultraviolet (UV) radiation is an effective means of decontaminating air and surfaces. Used in air-locks or as door barriers, UV radiation can isolate areas of differing levels of contamination within a building.[8] It is also useful for reducing extraneous contamination in laboratory and incubator rooms.

Window-type air conditioners used in infectious areas may be fitted with UV lamps to decontaminate recirculated air.[9] Similarly, a small UV air sterilizer may be used to sterilize air being discharged from aerated bacterial cultures or small aerosol chambers.[10]

Single sheets of paper may be decontaminated by an apparatus that rolls the paper slowly between UV lamps.[11]

Proper use of UV as a decontaminating agent requires an exact under-standing of its limitations. The radiation has no penetrating power and thus is most effective on exposed surfaces or in air. Proper concentration, contact time, and maintenance are also critical.

III. DECONTAMINATION PROCEDURES

A. GENERAL CONSIDERATIONS

The microbiological hazards arising from many common laboratory procedures are known,[12] and proper techniques will, to a large degree, reduce these hazards. However, it must be assumed that wherever bench-top work with infectious agents is carried out, surfaces within the work area will become contaminated with those agents. In addition, even the most well-ordered laboratory has an occasional accident. Infectious aerosols arising from either the routine manipulations or accidental occurrences may cause occupational illnesses and widespread contamination.

The hazard from microorganisms that can infect by the respiratory route may be particularly great, especially if the infectious agent possesses a high degree of environmental resistance. Lyophilized materials are also of concern since, in this state, even the forms ordinarily susceptible to environmental factors may be difficult to inactivate by ordinary means. The consequences or seriousness of the disease may also, to some extent, influence the decontamination procedures recommended. It should be remembered that no specific treatment exists for certain diseases, and it should be clear that even a "minor" disease agent should be handled in such a manner that no laboratory-acquired illnesses occur. Finally, the type of laboratory operation being conducted is significant. It is probable that accidents connected with the use of artificially cultured microorganisms are more hazardous than those connected with the handling of specimen material to be examined for the possible presence of pathogenic agents. However, there may be notable exceptions to such generalizations, e.g., the hazard of serum hepatitis from handling blood samples. It is obvious that the above factors and others must be carefully weighed when prescribing a decontamination procedure.

Another approach to the problem of preventing escape of pathogenic agents is that of enclosing microbiological operations in ventilated cabinets or cabinet systems. Culture materials spilled in a ventilated cabinet are usually well-contained and can be effectively decontaminated.

B. RESPIRATORY PROTECTION

Although it is important in the microbiological laboratory to practice good housekeeping and to decontaminate frequently to reduce chance contamination of culture materials, the primary purpose of decontamination following accidents or "spills" is to reduce or eliminate the infectious hazard to the workers involved. Therefore, the use of respiratory protection before and during decontamination must be considered. If the nature of a particular laboratory operation is such that "spills" are unavoidable or that accidents occur frequently, the laboratory director should insist on the continuous

wearing of effective respiratory protection. For less frequently occurring incidents, it may suffice to have such protective equipment available when needed.

Surgical gauze masks are not recommended. Studies have shown that their filtration efficiency against bacterial aerosols is very poor,[13,14] and the facial fit is also unsatisfactory.

Several types of commercial respirators,* face masks,** or ventilated personnel hoods*** are available that will offer adequate protection. In areas of extreme hazard, a complete ventilated suit*** may be worn, but the situation will rarely require such drastic measures.

C. DECONTAMINATION OF ROOMS AND BUILDINGS

1. Vapor Disinfectants

Vaporized formaldehyde has been used extensively as a space and surface decontaminant. It has been used for a number of years at Fort Detrick for decontaminating rooms, buildings, aerosol chambers, bacteriological cabinets, and filters. It is effective against bacteria, fungi, viruses, and ricksettsiae, as well as insects and other animal life.

The bactericidal efficiency of formaldehyde vapor is a direct function of the concentration, relative humidity, and temperature. A temperature of 75°F or higher is desirable, and the effectiveness of the disinfectant decreases rapidly as the relative humidity drops below 70 per cent.

Formaldehyde has a disadvantage in that it polymerizes readily on surfaces. The polymers are difficult to remove, and may require frequent washes and long waiting periods before treated areas are again usable. This disadvantage can be partially overcome by diluting a standard formalin solution (37 per cent HCHO) with methanol (five parts formalin solution to three parts methanol). Either the formalin solution or the formalin-methanol mixture may be used interchangeably for any of the applications described below.

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* Type R2000 Respirator with R-25 Filter, American Optical Co., South-
   bridge, Massachusetts; L800 All Dust Respirator with L-800-7 Filter,
   Pulmosan Safety Equipment Corporation, Brooklyn, New York; Dustfoe
   Ultra Filter Respirator, Catalog No. CM-77668, Mine Safety Appliances
   Co., Pittsburgh, Pennsylvania.
** Mine Safety Appliances Co., Pittsburgh, Pennsylvania.
*** Snyder Manufacturing Company, New Philadelphia, Ohio.

Beta-Propiolactone (BPL) has several advantages over formaldehyde as a vapor disinfectant:

(a) Its vapors are lachrymatory but are less irritating than those of formaldehyde.

(b) It does not readily polymerize on surfaces so that there is little or no residua.

(c) It acts more rapidly. However, in the liquid state, BPL is more toxic than formaldehyde, and in handling it, care must be taken that it does not contact the skin.

In using either formaldehyde or BPL, the room or building need not be hermetically sealed, but all exterior doors and windows must be closed except for those essential to the admission of the vapor. Interior doors should be open to allow free passage of the vapor. Forced ventilation should be shut off or reduced to a minimum, and where practicable, steam should be introduced to raise the humidity.

When treating a single room while the remainder of the building is in use, it is best to seal off any air supply ducts to the room and other openings to keep the fumes from seeping to other areas. Air exhaust ducts should not be sealed, so that a negative air pressure is maintained in the room during treatment. Often it is undesirable to use formaldehyde or BPL in buildings or rooms. The dual use of liquid germicides and ultraviolet radiations[8] is somewhat effective in decontaminating entire rooms.

2. Dissemination

a. Formaldehyde

Almost any method of dispersing formaldehyde into the air in suitable quantities is satisfactory for the use of this chemical as a space decontaminant. Since it is most efficient at higher temperatures and humidities, steam ejectors or steam vaporizers are most conveniently used for small areas. For large areas, a large-volume mechanical-type vaporizer is used. The formaldehyde solution is introduced in a concentration of one milliliter per cubic foot of space. In making this calculation, any air-flow through the space must be taken into account, and additional formaldehyde added to obtain the above concentration.

After a hold period of eight to ten hours (to allow for complete dispersion and adequate contact), doors and windows may be opened and forced ventilation resumed. Formaldehyde fumes are persistent, and a large building may require two to three water washes of the floor and two to three days' ventilation before normal entry. Casketing on refrigerators, freezers, etc. should be well cleaned of formaldehyde polymers and thoroughly aired to remove residual vapors that might subsequently damage stored

cultures. For smaller areas, a shorter hold period is required and over-night airing is generally sufficient. Formaldehyde is relatively noncorro-sive, and it can be generally assumed that any equipment or apparatus that will not be damaged by the necessary humidity will not be damaged by the formaldehyde.

b. Beta-Propiolactone

The technique for disseminating BPL is similar to that for formaldehyde. However, more care must be exercised to make certain that the BPL is well vaporized. The chemical must leave the disseminator as a vapor or in particles small enough so that they vaporize rapidly. Other-wise, the liquid droplets settle or impinge on surfaces and effective dissemination is not obtained. Liquid BPL is injurious to rubber items and painted surfaces if it is not immediately washed off. To allow a margin for leakage and condensation, BPL is disseminated in a concentration of one gallon for each 16,000 cubic feet of space. In making this calculation, any airflow through the space must be taken into account and additional BPL added to obtain the above concentration.

After a hold period of two to three hours, doors and windows may be opened and forced ventilation resumed. At this point, entry into treated areas should be made only with protective clothing and respiratory protection. Proper airing will generally allow normal entry after two to three hours.

c. Vaporizers

The following commercially available dispensers are suitable for vaporizing either formaldehyde or BPL:

(a) Challenger Model 5100 Vaporizer, manufactured by Z & W Machine Products Inc., Cleveland, Ohio. Suitable for rooms or small buildings.

(b) Hydro-Mist Vaporizer, Model H, manufactured by Arnold Laboratories, 7103 Laurel Canyon Blvd., North Hollywood, California. Suitable for rooms.

(c) Todd Insecticide Fog Applicator, Model 40 E, manufactured by Combustion Equipment Division, Todd Shipyard Corporation, Elmhurst, Long Island, New York. Suitable for large buildings.

These dispensers are used as single or multiple units, according to the size of the structure to be treated.

D. DECONTAMINATION OF CABINETS, CHAMBERS, AND OTHER CLOSED AREAS

Several practical decontaimination procedures are available that are suitable for different types of cabinets or chambers. Systems capable of withstanding full vacuum and pressure as high as 20 pounds per square inch gauge (psig) can be sterilized with steam or with Carboxide* gas (a commercial mixture of ten per cent ethylene oxide and 90 per cent carbon dioxide). Nonpressurized systems may be sterilized with formaldehyde and steam, beta-propiolactone or, if constructed of corrosion-resistant metal or plastic, with sprays of peracetic acid.

Bacteriological cabinets in which infectious organisms are handled are routinely decontaminated with formaldehyde. The Hydro-Mist Vaporizer or Penberthy X6-96, Series 1, steam ejector** are recommended, although any means of vaporizing is satisfactory. The cabinet should be closed as tightly as possible to reduce airflow to a minimum. Pressure-sensitive tape is useful in closing large cracks.

The formaldehyde solution is vaporized into the cabinet at a rate of one milliliter per cubic foot of airflow, plus one milliliter for each **cubic foot of space within the cabinet. Thus, if the cabinet contains 50** cubic feet and the airflow is 20 cubic feet per minute (cfm), then 650 milliliters of formaldehyde solution (20 x 1 x 30 + 50) must be vaporized in 30 minutes.

This technique will decontaminate the entire cabinet system; i.e., cabinet, exhaust filter, exhaust duct, and blower.

Unless a steam-activated vaporizer is used, the humidity must be raised by boiling water or injecting steam into the cabinet.

Ultraviolet lamps installed in cabinets and similar apparatus can be used to reduce air-borne contaminants, but should not be relied upon for complete sterilization. Satisfactory results can be obtained if ultraviolet radiation is combined with washing with an effective germicidal solution. Blowers or negative pressure systems attached to cabinets or chambers should ordinarily not be turned off until the decontamination is complete.

E. DECONTAMINATION OF BUILDING EXHAUST FILTERS

Laboratory buildings where infectious organisms are used extensively are often equipped with bacteriological filters for filtering exhaust air from the building ventilation systems. Periodically the filter media must be changed, and for this to be done safely, the filters are decontaminated with formaldehyde.

* Union Carbide and Carbon Chemicals Corp., New York, New York.
** Penberthy Injector Co., Detroit 2, Michigan.

The airflow through the exhaust plenum is reduced by damping the blower, and the building air supply is shut off to maintain a negative pressure in the exhaust plenum. Formaldehyde solution is disseminated into the plenum at the rate of one milliliter for each cubic foot of airflow for 30 minutes; i.e., if the airflow is 600 cfm, then 18,000 milliliters of formaldehyde solution (600 x 1 x 30) will be disseminated in 30 minutes. The most convenient means of doing this is with a steam ejector installed in the wall of the plenum on the contaminated side of the filters. In large plenums, proper concentration and distribution of formaldehyde vapor may require more than one injection site.

Stand-by personnel should be maintained inside the building to detect backwash of formaldehyde vapors. This is particularly important where exhaust ducts enter the bottom of the plenum, because condensed formaldehyde may flow down the ducts into the rooms.

Generally, the filters are allowed to dry overnight, after which they may be removed with minimum precautions.

F. DECONTAMINATION OF TABLE TOPS AND FLOORS

Contamination of exposed working surfaces and floors usually results from dropping or spilling infectious materials. With many infectious agents, the prime hazard to personnel is from air-borne particles that may result from an accident. When a spill occurs, it is best for personnel to leave the room immediately and close the door. Breathing of aerosolized particles can be avoided by holding the breath until out of the room. If the skin, clothing, or shoes of exposed persons have become contaminated, nontoxic liquid disinfectants should be applied. In any case, such persons should remove their clothing and shower thoroughly. The contaminated clothing may later by sterilized by autoclaving or by treatment with ethylene oxide gas. A method sometimes used for washable clothes is to place them in an automatic-type home washing machine with a suitable quantity of a quaternary ammonium solution.

If ultraviolet lamps are installed in the ceiling of the laboratory room,[8]/ these should be turned on for 30 minutes to inactivate any aerosol created by the spill. A portable high-intensity ultraviolet apparatus* such as that used for terminal disinfection of hospital rooms can be used if ceiling lamps are not available. If ultraviolet is not available, one hour should be allowed for air-borne particles to settle.

Next, a person wearing a respiratory protective device (Section III, B) should enter the room and apply generous amounts of liquid germicide. This should be applied gently, rather than by spraying vigorously, to avoid formation of additional aerosols.

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* Hanovia Chemical and Manufacturing Company, Newark, New Jersey.

The procedure outlined above cannot be expected to apply to all types of microorganisms but, in general, would be applicable to microorganisms of high infectivity such as the etiological agents of brucellosis and tularemia.

G. STERILIZATION OF SEWAGE AND OTHER LIQUID WASTES

From the standpoint of public health, it is usually not necessary to attempt central sterilization of all human excrement. In some infectious disease laboratories it may be important to sterilize or decontaminate excrement from infected animals or urine and fecal samples received for bacteriological analysis. If possible, animal debris should be sterilized in the animal cage, by autoclaving, before being removed. If this is not possible, the cage debris should be soaked with a liquid disinfectant before being scraped out of the cage.

In research institutions where considerable quantities of infectious microorganisms are discharged in liquid wastes, it may be desirable to collect these in a storage tank and subsequently sterilize them with steam. **Such a waste-treatment tank may be operated on a continuous pasteurization basis or on a batch basis.**

Since most infectious organisms released by accidents or spills may eventually find their way to sink or floor drains in the laboratory, it is recommended that disinfectant solution or water be poured into all drains at regular intervals to keep the traps full and prevent backflow of gases or microbiological contamination.

H. STERILIZATION OF EQUIPMENT AND APPARATUS WITH ETHYLENE OXIDE

The ideal sterilizing agent might be fairly well described as an inexpensive, nontoxic and noncorrosive gas that is highly penetrating at low pressures, exhibits a rapid lethal action against microorganisms, and is nonflammable in mixtures with air. Such an agent may never be found, but if it is, it will probably have many of the characteristics of ethylene oxide.

Ethylene oxide has been in routine use at Fort Detrick for a number of years. It has been used at times in its pure form, which requires special handling because it is so explosive in air mixtures. It has been used in mixtures with carbon dioxide or nitrogen, which requires that it be used under pressure. Its most extensive use today is in the form of a low-pressure mixture with two of the chlorofluorohydrocarbons (Freons) in a disposable 16-ounce can.* In this form it is a highly practical and convenient tool for increasing the usefulness of the laboratory autoclave.

* "Ethylene Oxide Mixture," Pennsylvania Engineering Co., Philadelphia, Pennsylvania.

Any steam autoclave can be inexpensively converted to its use without interfering with the use of the autoclave with steam. It enables the laboratory worker to sterilize many heat-labile substances or complex mechanical or electronic equipment that cannot be sterilized in any other way. Figure 1 shows an autoclave modified for use with the ethylene oxide mixture. This modification requires a small amount of piping and the attachment of a can-puncturing device for releasing the gas. One can of the mixture contains sufficient ethylene oxide to sterilize materials placed in a small laboratory autoclave (six-cubic-foot volume). By manifolding several openers, several cans can be discharged simultaneously - a convenient arrangement for using the cans on large autoclaves.

Figure 1 shows the laboratory vacuum line connected to the chamber. This is not essential, but improves the operation in two ways. Evacuating the chamber before admission of the gas aids in penetration and reduces losses of gas by leakage from a chamber that is not gas-tight. If the six-cubic-foot chamber is evacuated to 18 inches of mercury negative pressure, one can will return the chamber to atmospheric pressure.

A definite drawback to the use of ethylene oxide is the required exposure time. In concentrations practical for use, a minimum of six hours is required to sterilize materials contaminated with bacterial spores, and longer (overnight) exposures are recommended for routine use.

The autoclave can be similarly used with Carboxide, as seen in Figure 2. Here, after evacuation of the chamber, the gas is admitted to a positive pressure of 20 psig. The chamber must be sufficiently gas-tight to maintain this pressure overnight. This system is rendered much safer by using an expansion tank to prevent overpressurization of the autoclave.

Undiluted ethylene oxide gas may be used under certain conditions as a sterilizing agent, but this is not recommended as a routine procedure because of the explosion hazards. For example, in an emergency, certain equipment can be decontaminated in an open field, using a gas-tight bag. The apparatus to be sterilized is placed in the bag in an open container. The bag is tightly sealed and the liquid allowed to evaporate. The exposure time should be about 16 hours at a temperature above the boiling point of the liquid (11°C).

Clothing or wearing apparel decontaminated with ethylene oxide should always be thoroughly aired for 24 hours before re-use because of the irritating action of ethylene oxide on human tissues. This gas is readily absorbed by rubber.

Experimental evidence indicates that ethylene oxide does not effectively inactivate smallpox virus in the presence of egg tissue, and it is not recommended for routine use. This suggests a similar possibility for agents yet untested.
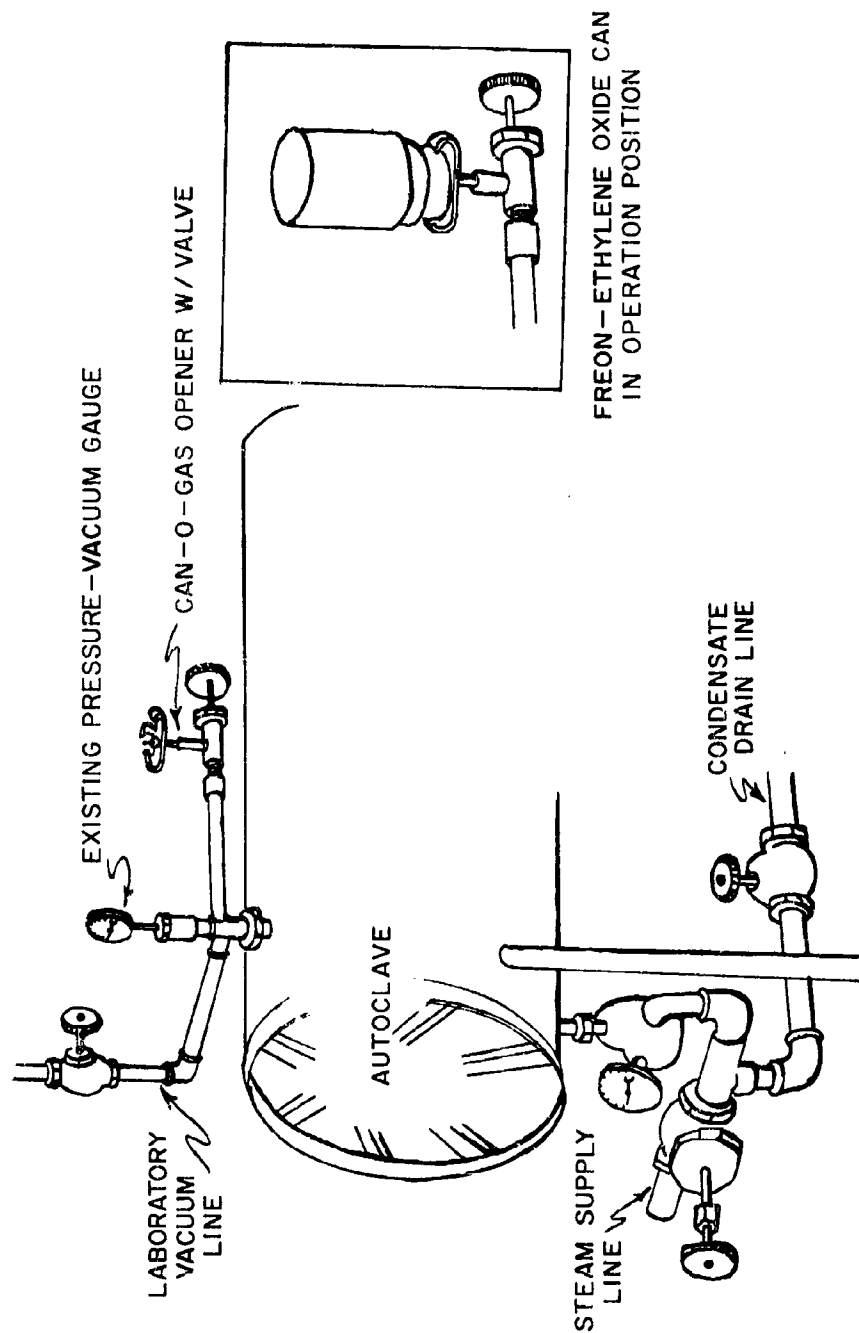
Figure 1. Modification of an Autoclave for Use with Cans of Freon - Ethylene Oxide. NOTE: A baffle must be installed where the gas enters the chamber. In double-door autoclaves where no baffle exists, the gas mixture must enter the jacket, not go directly into chamber.
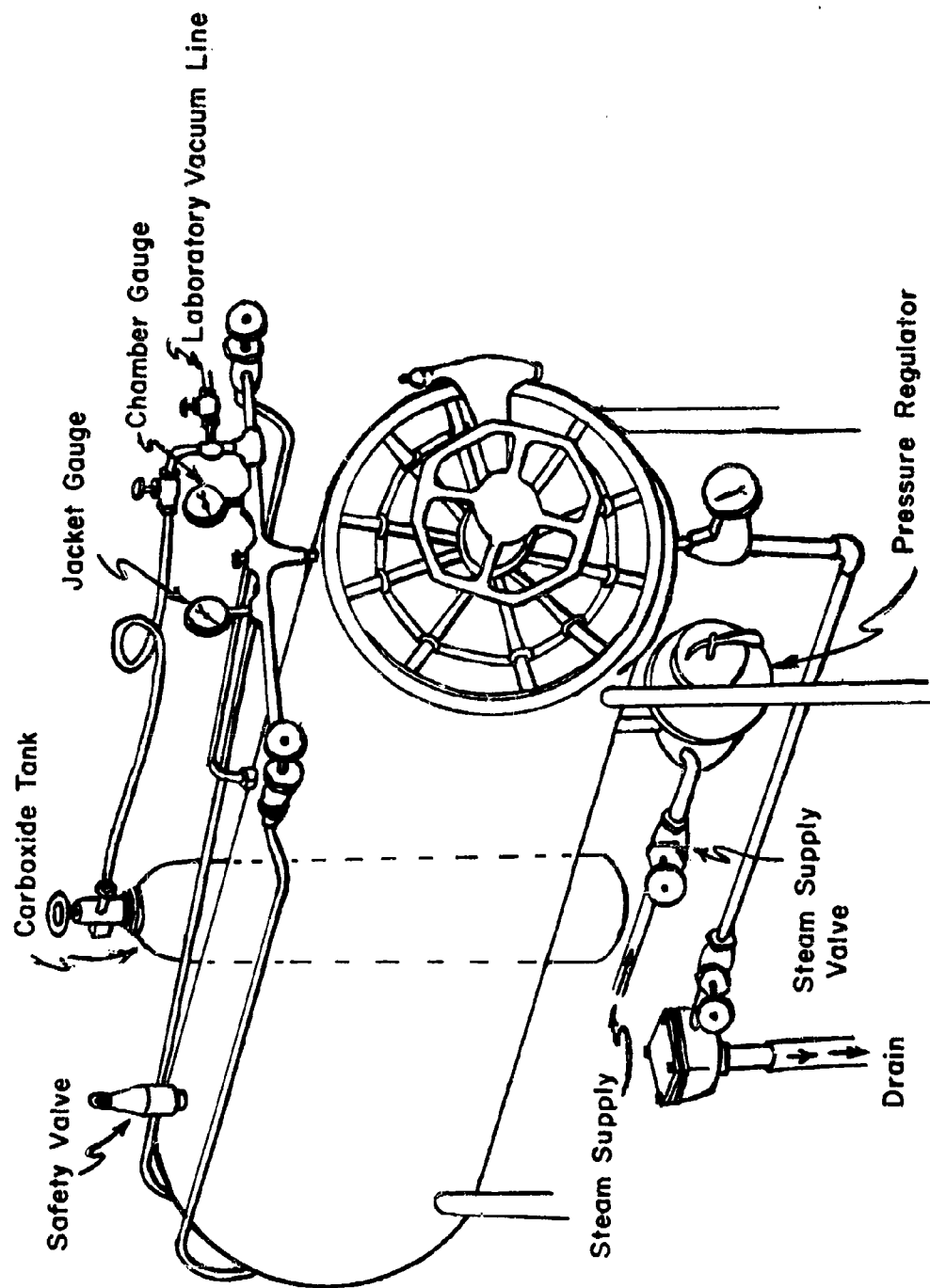
Figure 2. Modification of an Autoclave for Use with Carboxide Gas. NOTE: Autoclave door and safety valve must be capable of withstanding 20 psig of dry gas. An adaptor is needed for connection to the Carboxide tank.

I. DECONTAMINATION OF PAPER

Paper used in the infectious laboratory for recording data may accidentally or unknowingly become contaminated. It is best to decontaminate such paper routinely before it is taken out of the laboratory or handled by persons who may be unaware of its potential hazard. This paper can be sterilized by autoclaving or treating with ethylene oxide. An alternate method applicable to the sterilization of single sheets of paper in the laboratory area utilizes ultraviolet radiation.[11]/

J. DECONTAMINATION OF INCUBATORS AND REFRIGERATORS

Accidents frequently occur while infectious materials are being held at incubation or refrigeration temperatures, thus necessitating decontamination of the room or apparatus. Cultures incubating on shaking machines or cultures being subjected to aeration are particularly hazardous because they are left unattended for many hours. Cultures stored in refrigerators or freeze chests are often upset or broken, or the stoppers may fall out of flasks or bottles. Dropping Petri plates on the floor creates hazards to which particular attention must be paid.[15]/ Pressurized spray devices should not be used to decontaminate broken Petri plates or other broken glassware.

Non-walk-in-type incubators and refrigerators used for infectious materials should be periodically cleaned and wiped out with a liquid disinfectant. If the apparatus is not overcrowded and the contents are kept in an orderly fashion, accidents due to dropping materials will be decreased.

Walk-in incubators can be equipped with ceiling ultraviolet lamps. Constant irradiation of the interior will reduce aerosols resulting from flasks breaking on shaking machines and similar accidents.[8]/

K. DECONTAMINATION OF PERSONNEL

Exact procedures for decontaminating personnel after accidents with infectious materials are difficult to prescribe because the degree of contamination, type of agent, and other factors will vary widely. It is important, however, that all persons handling disease-producing organisms be taught to be constantly aware of what they touch or handle. Skin decontaminants should be used frequently during laboratory procedures. If possible, clothing and shoes worn in the laboratory should not be worn outside the laboratory. After working with infectious microorganisms, and particularly after known accidents, persons should shower thoroughly and wash with a soap containing a disinfectant such as hexachlorophene. Washing the hair, along with the wearing of caps in the laboratory, is recommended.

L. DECONTAMINATION OF ANIMAL ROOMS

Procedures in Animal Rooms depend somewhat upon what other protective measures are used. Unless infected animals are held in sealed cages, respiratory devices should always be worn in the animal room. It may be desirable for all persons leaving the animal room to decontaminate their shoes. A sponge-rubber pad soaked with a liquid disinfectant can be used for this purpose. The use of ultraviolet cage racks[16] is recommended. Animal-Room floors should be washed with a liquid disinfectant daily and after each accident.

IV. PREVENTIVE DECONTAMINATION

The need for continuous and extensive decontamination measures in the infectious disease laboratory can often be greatly reduced by careful techniques and the proper use of safe equipment. Carrying out the majority of agent manipulations in a ventilated safety cabinet usually restricts infectious contamination to a safe area. Orientation lectures and training films may be useful for acquainting laboratory personnel with safe practices.

Laboratory regulations pertaining to safety may be organized in the form of a safety manual. General rules concerning entrance and exit of materials, eating, smoking, showering, changing clothes, procedures in case of accident, decontaminants to be used, and entrance of visitors should be clearly designated. Specific regulations covering certain operations such as tissue grinding, centrifuging, pipetting, and the use of needle and syringe should be given, as well as rules applying to certain areas such as the animal rooms.

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